

Teruko NAKAMURA\*, Jiro ENDO\* & Motoo NAGASAWA\*:  
**Geographical variation in essential oil composition  
of *Asiasarum sieboldii* (Aristolochiaceae)**

中村輝子\*・遠藤次郎\*・長沢元夫\*: ウスバサイシンの  
精油組成の地理的変異

*Asiasarum sieboldii* (Miq.) F. Maekawa is distributed in China, Korea and Japan and used for medicinal purposes. Several reports have indicated that the essential oil of this species consists of the phenylpropanoids and the monoterpenoids (Takahashi 1930, Nagasawa 1961, Saiki 1967), and that its composition varies geographically (Nagasawa 1961). However, at that time, it was difficult to examine the variation precisely. On the other hand, our recent investigations of the essential oils of *Asiasarum heterotropoides* (Fr. Schm.) F. Maekawa and *Asiasarum dimidiatum* (F. Maekawa) F. Maekawa demonstrated their geographical variations clearly (Nakamura *et al.* 1979, 1982).

As a consequence of the above findings, the essential oil composition of *A. sieboldii* from various districts of Japan was re-examined by gas chromatography (GC) in order to confirm its variational feature and to discuss the chemosystematics of the genus *Asiasarum*. The present study clarified the geographical variation within the species with regard to the following aspects: 1) the relative amount of the phenylpropanoids and the monoterpenoids, 2) the monoterpene pattern, and 3) the phenylpropanoid pattern. The first two aspects are described in this paper.

**Materials and methods** Materials were collected as previously reported (Nakamura *et al.* 1979). Their localities are shown in Fig. 4. Experimental methods and procedures except GC analyses were essentially identical with those in our previous paper (Nakamura *et al.* 1979). The GC analyses were performed as follows: (1) Hitachi 164-FID; glass capillary column, 30000×0.25 mm, PEG-20M; carrier gas, He; column temperature 140°C; and (2) Hitachi 164-TCD; stainless steel column, 2000×3 mm, PEG-20M (60–80 Mesh); carrier gas, He;

\* Faculty of Pharmaceutical Sciences, Science University of Tokyo, Shinjuku-ku, Tokyo 162. 東京理科大学 薬学部.

column temperature, 190°C.

**Results** The following components were found in the essential oil of *A. sieboldii*: phenylpropanoids—estragol, eugenol, methyleugenol, elemicin, euasaron, safrole, croweacin and asaricin; monoterpenoids—linalool, 1,8-cineol, terpinenol-4,  $\alpha$ -terpineol, eucarvone, carendione and borneol; orcinol derivatives—3,5-dimethoxytoluene, 3,4,5-trimethoxytoluene and 2,3,5-trimethoxytoluene; and an aliphatic hydrocarbon, *n*-pentadecane. The phenylpropanoids and/or the monoterpenoids.

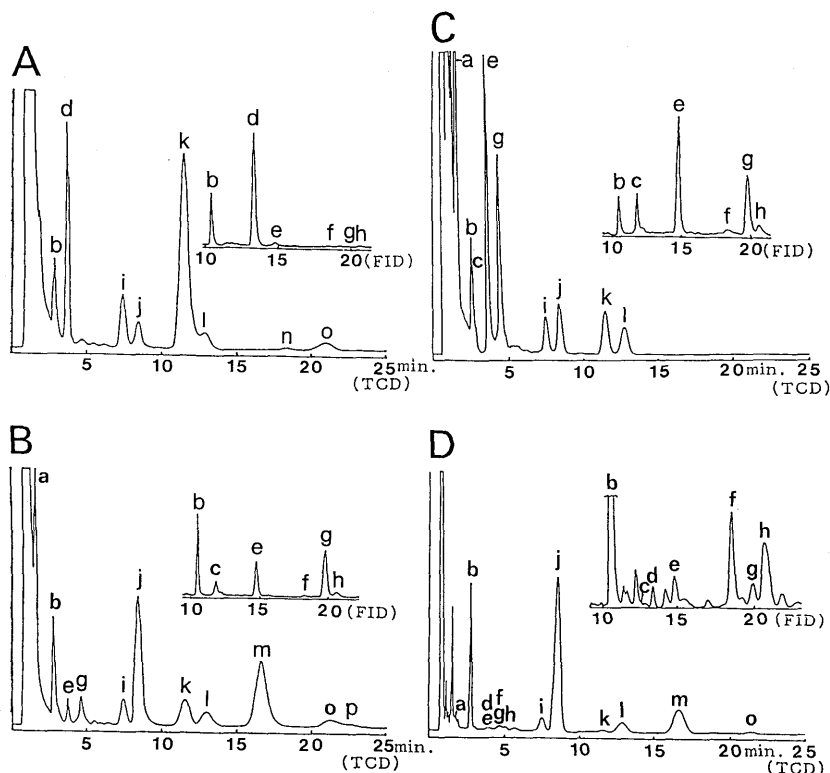


Fig. 1. Gas chromatograms of the essential oils from various locations of *A. sieboldii*. A: Mt. Tanigawa (Gunma Pref.). B: Sekigahara (Gifu Pref.). C: Mt. Tamamine (Shimane Pref.). D: Mt. Kura (Kumamoto Pref.). a: 1,8-cineol. b: *n*-pentadecane. c: linalool. d: eucarvone. e: terpinenol-4. f: estragol. g:  $\alpha$ -terpineol. h: borneol. i: 3,5-dimethoxytoluene. j: safrole. k: methyleugenol. l: 2,3,5- & 3,4,5-trimethoxytoluene. m: croweacin. n: eugenol. o: asaricin. p: elemicin. TCD: stainless steel column, 2000 $\times$ 3 mm, PEG-20M; carrier gas, He; column temperature, 190°C. FID: glass capillary column, 30000 $\times$ 0.25 mm, PEG-20M; carrier gas, He; column temperature, 140°C.

were present as the main constituents.

A compositional variation in essential oils of *A. sieboldii* was observed (Fig. 1). Most components varied, but the variations in the aliphatic hydrocarbon and the orcinol derivatives were not correlated with geographic distribution. The geographic variation in the phenylpropanoid pattern will be reported separately, as it is very complicated. The other chemical variation is reported below.

1) Geographical variation in relative amounts of phenylpropanoids and monoterpenoids. The relative amounts of phenylpropanoids and monoterpenoids in the essential oil were calculated as follows. Each peak area in the gas chromatogram was measured. The ratio of the sum of the peak areas of the phenylpropanoids to the total of those of the phenylpropanoids and the monoterpenoids was calculated. This value was expressed as the phenylpropanoid percentage in the essential oil.

The phenylpropanoid percentage of *A. sieboldii* showed individual variation even in the same population (Fig. 2). However, the range of the intrapopulation variation was not very wide. Samples having the phenylpropanoids as the main constituent and those containing the monoterpenoids as the main constituent did not coexist in the same population.

The phenylpropanoid percentages were compared in 110 populations (Fig. 3). The average percentages of the phenylpropanoids in each population are shown below. The number of samples examined is shown in parentheses. In populations marked with an \* samples were used after cultivation, and in those marked with \*\* dry materials were used. Aomori Pref.—Okuyagen, 73.9 (5); Mt. Eboshi, 62.2 (3); Mt. Hakkoda, 81.4 (3); Oirase, 69.9 (3); Mt. Iwaki, 89.3 (9). Akita Pref.—Mt. Taihei, 69.1 (2). Iwate Pref.—Mt. Ushigata-1, 67.6 (3); Mt. Ushigata-2, 70.1 (2). Yamagata Pref.—Mt. Gassan 68.8 (1); Yutagawa, 72.1 (10); Atsumi, 83.6 (3); Mt. Zao, 85.2 (12); Namerakawa, 85.3 (1). Fukushima Pref.—Mt. Ryozen-1, 86.4 (3); Mt. Ryozen-2, 81.9 (3); Tsuchiyu-1, 87.9 (3); Tsuchiyu-2, 86.7 (10); Mt. Bandai, 83.5 (2); Ushiogawa, 68.1 (3); Tanakura, 88.8 (3); Hinoemata, 80.3 (2); Mt. Hiuchi-1, 80.8 (2); Mt. Hiuchi-2, 92.1 (2). Ibaragi Pref.—Hanazono, 66.7 (3); Hananuki\*, 70.6 (1); Mt. Tsukuba, 82.2 (10). Tochigi Pref.—Sandogoya, 69.9 (3); Nasunishiiwasaki, 72.5 (11); Kawaji, 77.2 (3); Ryuzunotaki-1, 72.5 (3); Ryuzunotaki-2, 80.2 (2); Nikko Akazawa, 82.5 (14); Nikko Hanaishi, 59.3 (17); Ozasa, 75.2 (11); Sagebu, 75.0 (2); Mt. Kogashi, 79.9 (3). Gunma Pref.—Yunokoya, 77.7 (3); Mt. Tanigawa-1, 82.6 (10); Mt. Tanigawa-2,

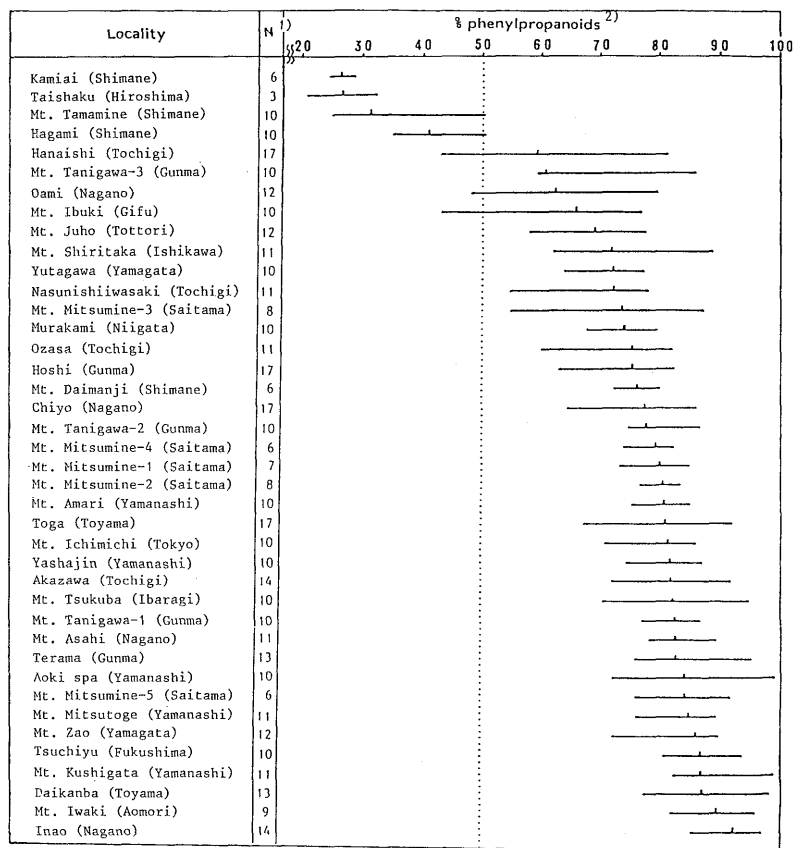


Fig. 2. Intra- and interpopulational variation in relative amounts of phenylpropanoids (P) and monoterpenoids (M) in the essential oil of *A. sieboldii*. 1) number of samples examined. 2)  $100 \times P/P+M$ ; P, sum of peak areas of phenylpropanoids in a gas chromatogram; M, that of monoterpenoids.

77.7 (10); Mt. Tanigawa-3, 60.8 (10); Hoshi, 75.3 (17); Higashiogawa, 87.1 (3); Terama, 82.8 (13); Mt. Akagi, 68.2 (3). Saitama Pref.—Mt. Mitsumine-1, 80.0 (7); Mt. Mitsumine-2, 80.5 (8); Mt. Mitsumine-3, 73.9 (8); Mt. Mitsumine-4, 79.5 (6); Mt. Mitsumine-5, 84.1 (6). Tokyo Pref.—Mt. Ichimichi, 81.4 (10). Kanagawa Pref.—Himetsugi, 84.3 (3). Niigata Pref.—Murakami, 74.4 (10); Mt. Yahiko, 76.4 (1); Sado Tochu, 58.9 (5); Sado Yoneyama, 61.2 (2). Toyama

Pref.—Toga, 81.0 (17); Daikanba, 86.9 (13); Kamihira, 70.4 (2); Goka, 81.2 (3). Ishikawa Pref.—Mt. Shiritaka, 71.9 (11). Yamanashi Pref.—Mt. Koma, 89.5 (3); Aoki Spa-1, 84.1 (10); Aoki Spa-2, 72.3 (5); Aoki Spa-3, 70.9 (3); Mt. Amari, 80.9 (10); Yashajin, 81.9 (10); Mt. Kushigata, 86.7 (11); Mt. Mitsutoge, 84.9 (11); Mt. Hichimen-1, 71.0 (3); Mt. Hichimen-2, 65.5 (3). Nagano Pref.—Nonomi, 85.8 (3); Mt. Madarao-1, 82.9 (5); Mt. Madarao-2, 84.4 (3); Mt. Kurokura, 85.6 (3); Oami, 64.4 (12); Warabidaira-1, 66.3 (3); Warabidaira-2, 68.4 (2); Togakushi, 86.6 (3); Jigokudani, 75.7 (3); Happoone, 73.6 (3); Futamata, 73.9 (2); Koda, 91.4 (5); Kamishiro, 75.2 (3); Mt. Asahi, 82.6 (11); Inao, 92.0 (14); Mt. Kamuriki, 80.9 (3); Kamikochi, 79.6 (2); Sanjiro, 84.5 (3); Tobira, 87.6 (3); Mt. Nyugasa, 65.5 (3); Obugawa, 91.6 (3); Inakita, 75.6 (5); Chiyo, 77.5 (17). Gifu Pref.—Hirayu, 65.6 (3); Mt. Ibuki, 65.7 (10). Mie Pref.—Mt. Fujiwara, 77.8 (3). Hyogo Pref.—Mt. Ooginosen, 81.6 (3); Tottori Pref.—Mt. Juho, 69.0 (12). Shimane Pref.—Mt. Daimanji-1\*, 76.0 (2); Mt. Daimanji-2\*, 76.2 (1); Mt. Tamamine, 31.3 (10); Kamiai-1\*, 27.3 (3); Kamiai-2\*, 24.7 (3); Hagami, 40.9 (10). Hiroshima Pref.—Tojo\*, 48.0 (2); Taishaku\*, 26.6 (2). Nagasaki Pref.—Mt. Mitake, 92.7 (3); Kaidokoro, 94.8 (3). Kumamoto Pref.—Mt. Kura, 98.7 (10); Matoishi\*\*, 99.0 (3).

In 98 populations, the phenylpropanoid percentages were over 65%, while in 6 populations they were under 50%. These 6 populations having the monoterpenoids as their main constituent were found in the inland of Shimane and Hiroshima Prefectures.

2) Geographical variation in the monoterpenoid pattern. The monoterpenoid pattern was uniform in the same population. However, geographical variations were evident. Three different monoterpenoid patterns were found within *A. sieboldii* from Japan (Fig. 1, Fig. 4).

The first pattern was

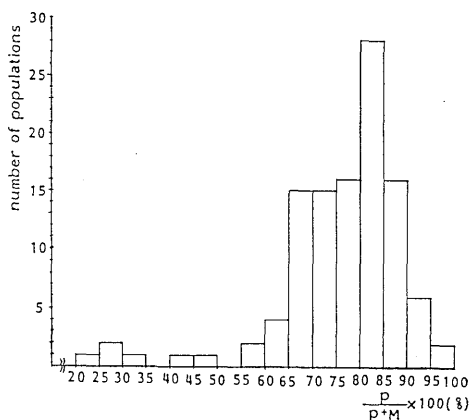


Fig. 3. Intropopulational variation in relative amounts of phenylpropanoids (P) and monoterpenoids (M) in the essential oil of *A. sieboldii*. The average value of phenylpropanoid percentage in each population is shown.

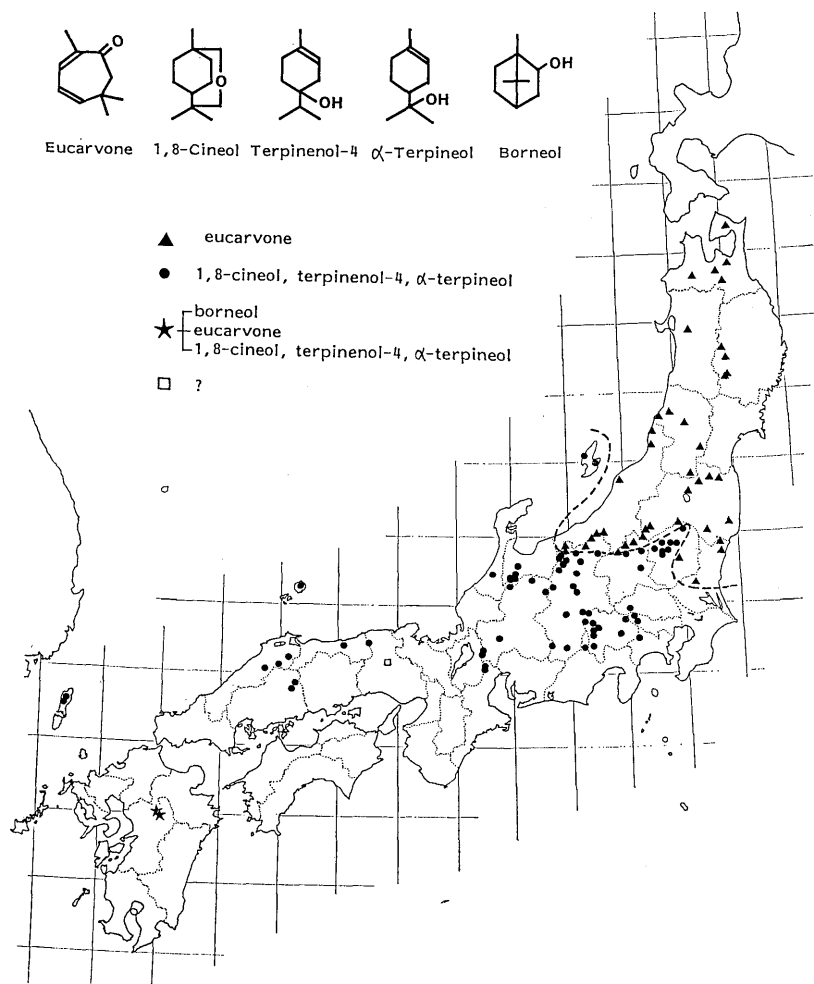


Fig. 4. Geographical variation in the monoterpenoid pattern of *A. sieboldii*.

characterized by the predominance of eucarvone and traces of 1,8-cineol,  $\alpha$ -terpineol, terpinenol-4 and borneol (Fig. 1-A). This pattern was found in the following populations (Fig. 4). In a population marked with an \*, samples were used after cultivation. Aomori Pref.—Okuyagen, Mt. Eboshi, Mt. Hakkoda, Oirase, Mt. Iwaki; Akita Pref.—Mt. Taihei; Iwate Pref.—Oshuku, Nishinamari,

Mt. Ushigata-1, Mt. Ushigata-2; Yamagata Pref.—Mt. Gassan, Yutagawa, Atsumi, Mt. Zao, Namerakawa; Fukushima Pref.—Mt. Ryozen-1, Mt. Ryozen-2, Mt. Shinobu, Tsuchiyu-1, Tsuchiyu-2, Mt. Bandai, Ushiogawa, Tanakura, Hinoemata, Mt. Hiuchi-1, Mt. Hiuchi-2; Ibaragi Pref.—Hanazono, Hananuki\*, Mt. Tsukuba; Tochigi Pref.—Sandogoya, Mt. Kogashi; Gunma Pref.—Yunokoya, Mt. Tanigawa-1, Mt. Tanigawa-2, Mt. Tanigawa-3, Hoshi; Niigata Pref.—Gatsugi, Murakami, Mt. Yahiko; Nagano Pref.—Nonomi, Mt. Kurokura, Mt. Kuroiwa, Mt. Madarao-1, Mt. Madarao-2, Oami, Warabidaira-1, Warabidaira-2.

The second pattern was distinguished by the predominance of 1,8-cineol,  $\alpha$ -terpineol and terpinenol-4, the small content of borneol, and a trace of eucarvone (Fig. 1-B, Fig. 1-C). The following samples showed this pattern (Fig. 4). In populations marked with an \*, samples were used after cultivation. Tochigi Pref.—Nasu Nishiiwasaki, Happogahara, Kawaji, Ryuzunotaki-1, Ryuzunotaki-2, Nikko Akazawa, Nikko Hanaishi, Ozasa, Sagebu; Gunma Pref.—Higashiogawa, Terama, Mt. Akagi; Saitama Pref.—Mt. Mitsumine-1, Mt. Mitsumine-2, Mt. Mitsumine-3, Mt. Mitsumine-4, Mt. Mitsumine-5; Tokyo Pref.—Nippara, Mt. Ichimichi; Kanagawa Pref.—Himetsugi; Niigata Pref.—Sado Tochu, Sado Yoneyama; Toyama Pref.—Toga, Daikanba, Kamihiro, Goka; Ishikawa Pref.—Mt. Shiritaka; Yamanashi Pref.—Kosuge, Mt. Koma, Aoki Spa-1, Aoki Spa-2, Aoki Spa-3, Mt. Amari, Yashajin, Mt. Kushigata, Mt. Mitsutoge, Mt. Hichimen-1, Mt. Hichimen-2; Nagano Pref.—Togakushi, Jigokudani, Happon, Futamata, Koda, Kamishiro, Mt. Asahi, Inao, Mt. Kamuriki, Kamikochi, Sanjiro, Tobira, Mt. Nyugasa, Obugawa, Inakita, Chiyo; Gifu Pref.—Utsubo, Shirakawa, Hirayu, Kitayama, Mt. Ena, Mt. Ibuki, Sekigahara; Shizuoka Pref.—Nakanoshuku; Mie Pref.—Mt. Oike, Mt. Fujiwara; Hyogo Pref.—Mt. Ooginosen; Tottori Pref.—Mt. Juho; Shimane Pref.—Mt. Daimanji-1\*, Mt. Daimanji-2\*, Mt. Tamamine, Kami-1\*, Kami-2\*, Hagami; Hiroshima Pref.—Tojyo\*, Taishaku\*; Nagasaki Pref.—Mt. Mitake, Kaidokoro.

The third monoterpenoid pattern was characterized by the predominance of borneol and small amounts of 1,8-cineol,  $\alpha$ -terpineol, terpinenol-4 and eucarvone (Fig. 1-D). This pattern was found in the samples from Mt. Kura and Matoishi in Kumamoto Prefecture (Fig. 4). In Matoishi, dry materials were used.

The monoterpenoid pattern in the samples from Uogataki in Hyogo Prefecture was different from the above-mentioned three patterns and has not yet been determined.

As described above, the populations from Honshu could be classified into two groups according to their monoterpenoid patterns. The samples from the north of the border line, located between 36° and 37°N, had the first monoterpenoid pattern, while those from the south of the line had the second monoterpenoid pattern (Fig. 4).

**Discussion** As a result of these investigations, the geographical variation in the essential oil of *A. sieboldii* was determined with respect to the two chemical characters described above. The variational features of these characters were different from each other.

From the variation in the relative amount of the monoterpenoids and the phenylpropanoids, it is apparent that the phenylpropanoids are the main components in most of the samples, and that the monoterpenoids are the main components in the samples from limited areas. This variational feature is similar to those of *A. heterotropoides* and *A. dimidiatum* previously reported (Nakamura *et al.* 1979, 1982). These results suggest that this chemical character is not very important in the chemosystematics of *Asiasarum*. As discussed before (Nakamura *et al.* 1979, 1982), it is supposed that the main components of the essential oil of *Asiasarum* may originally be phenylpropanoids and that phenylpropanoids may have been replaced by monoterpenoids as the main constituents in some samples.

Early investigations of the geographical variations in monoterpenoid constituents of *A. sieboldii* indicated the following points. The eucarvone content gradually increased as the sample location shifted toward the north, while it gradually decreased as the sample location shifted toward the southwest. The geographical variation in 1,8-cineol was completely contrary to that in eucarvone (Nagasawa 1961). However, the present study has shown that the geographical variation in the monoterpenoid pattern is not clinal but discontinuous. Fujita (1966) made the following comment on the report of Nagasawa (1961). "The samples from the north of the Fossa Magna were characterized by the presence of eucarvone and the absence of 1,8-cineol, while those from the south of the Fossa Magna were characterized by the absence of eucarvone and the presence of 1,8-cineol." However, the present study has shown that the Fossa Magna is not the border line dividing *A. sieboldii* into two groups and that the samples from Kyushu have another monoterpenoid pattern.

The present results have also proposed that *A. sieboldii* can be divided into

three groups according to the monoterpenoid pattern and that this chemical character is an important systematic criterion within the genus *Asiasarum*. The monoterpenoid pattern of eucarvone found in *A. sieboldii* from the north of the border line (Fig. 4) is identical with that of *A. heterotropoides* distributed in Hokkaido and the Tohoku districts (Nakamura *et al.* 1979), while the monoterpenoid pattern of 1,8-cineol,  $\alpha$ -terpineol and terpinenol-4, which was found in *A. sieboldii* from the south of the border line (Fig. 4), agrees with that of *A. dimidiatum* distributed in Kyushu, Shikoku and the Kii Peninsula (Nakamura *et al.* 1982). From these facts, we conclude that the former monoterpenoid pattern is characteristic of the northern taxa of Japanese *Asiasarum* and that the latter is distinctive of some southwestern taxa of Japanese *Asiasarum*. Another monoterpenoid pattern of borneol, eucarvone, 1,8-cineol,  $\alpha$ -terpineol and terpinenol-4, which was detected in the samples from Kumamoto Prefecture (Kyushu), resembles that of Chinese and Korean plants of *Asiasarum* (Pan *et al.* 1984; Nakamura unpublished). These facts suggest that the samples from Kumamoto are related to those from China and Korea.

In the course of the present study, we found that the variation in the monoterpenoid pattern is correlated with that in morphological characters. These details will be reported separately.

We are grateful to Prof. Mikio Ono of Makino Herbarium, Tokyo Metropolitan University for his valuable advice and to the many persons who kindly helped us in field collections or provided the materials to us. We are indebted to Ms. Mary McMenamin of National Institutes of Health, USA for her linguistic check.

### References

- Fujita, Y. 1966. Classification and phylogeny of the genus *Asarum* viewed from the constituents of essential oil. Bot. Mag. Tokyo 79: 783-790. Nagasawa, M. 1961. Analysis of crude drugs by infrared spectrophotometry II. YAKUGAKU ZASSHI 81: 129-138. Nakamura, T., J. Endo & M. Nagasawa 1979. Geographical variation of the essential oil components in *Asiasarum heterotropoides*. Journ. Jap. Bot. 54: 334-341. —, — & T. Hamada 1982. Variation of *Asiasarum dimidiatum*. Journ. Jap. Bot. 57: 366-376. Pan, Xu, Wang, Yang & Zhang 1984. GC/MC Analysis of the essential oils from plants of genus

*Asarum* in China. Bull. Chin. Mat. Med. 9: 27-29; 175-177. Saiki, Y. 1967. Gas chromatographical studies on natural volatile oils III, IV, V. YAKUGAKU ZASSHI 87: 1535-1538 1539-1543, 1544-1547. Takahashi, T. 1930. Essential Oil of *Asarum Sieboldi* Miq. (Aristolochiaceae). Nippon Kagaku Kaishi 51: 432-441.

\* \* \* \*

ウスバサイシンの精油組成の地理的変異を明らかにするとともに、ウスバサイシン属植物を化学成分の面から考察した。精油の主成分は、ほとんどの検体ではフェニルプロパン類であり、一部の地域のものではモノテルペン類である。この地理的変異の傾向はオクエゾサイシンやクロフネサイシンで報告した結果にはほぼ類似している。また、モノテルペン類の組成にみられた地理的変異は明瞭であり、これによって、日本に自生するウスバサイシンを次の 3 群に分けることができる。本州中部以北産のウスバサイシンはユーカルボンに富み、微量の 1,8-シネオール、 $\alpha$ -ターピネオール、ターピネノール-4、ボルネオールを含む。一方、本州中部以西産のもの（熊本県のを除く）は 1,8-シネオール、 $\alpha$ -ターピネオール、ターピネノール-4 に富み、微量のユーカルボンとボルネオールを含む。また、熊本県のはボルネオールに富み、少量のユーカルボン、1,8-シネオール、 $\alpha$ -ターピネオール、ターピネノール-4 を含む。モノテルペン類の組成の面では、本州中部以北のウスバサイシンはオクエゾサイシンに、本州中部以西のウスバサイシンはクロフネサイシンに一致し、また、熊本県のものは中国大陆や朝鮮半島に分布する近縁種に類似している。後にウスバサイシンの形態の変異を報告する際に、今回指摘した化学成分の差異の分類学的意義も述べる予定である。

□相馬研吾・安田啓祐：植物の採集と観察 194 pp. 1986. 講談社，東京．¥2,600. 維管束植物について観察の仕方を丁寧に指導する手引書で，対象は大学生程度である。よく撮れて印刷もはっきりした写真がふんだんに使われ，約半分が写真ページになっている。それは B 5 判の紙面一ぱいに 10 数個の写真を並べて構成され，詳しい解説と対向している。シダ植物では 70 種以上が取扱われ，幼植物・成植物の生態，孢子囊群など各部分の拡大，孢子・前葉体・染色体と揃っていて，シダの入門書としてもわかりやすくできている。日本産のシダ 154 種類の孢子の採集適期と採集可能期を示した孢子カレンダーは初めての試みで便利なものである。種子植物は代表的なものに限られて数は少ないが，花の解剖が主となり特に子房の中の胚珠の様子などは写真の強みを発揮している。むずかしいイチョウの配偶体もきれいに撮れている。（伊藤 洋）